Plasmid-Mediated Novel *bla*$_{NDM-17}$ Gene Encoding a Carbapenemase with Enhanced Activity in a ST48 *Escherichia coli* Strain

Zhihai Liu[a], Yang Wang[a], Timothy R. Walsh[b], Dejun Liu[a], Zhangqi Shen[a], Rongmin Zhang[a], Wenjuan Yin[a], Hong Yao[a], Jiyun Li[a], Jianzhong Shen[a#]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China

[b] Department of Medical Microbiology and Infectious Disease, Cardiff Institute of Infection & Immunity, UHW Main Building, Heath Park Hospital, Cardiff CF144XN

Keywords: NDM-17, carbapenemase, ST48, IncX3

Running title: NDM-17 with enhanced carbapenemase activity

 # Address correspondence to Jianzhong Shen, sjz@cau.edu.cn

ABSTRACT

Carbapenem-resistant Enterobacteriaceae (CRE) have spread worldwide, leaving very few treatment options available. New Delhi metallo-beta-lactamase (NDM) is the main carbapenemase mediating CRE resistance, and is of increasing concern. NDM-positive Enterobacteriaceae of human origin are frequently identified; however, the emergence of NDM, particularly novel variants, in bacteria of food animal origin has never been reported. Here, we characterize a novel NDM variant (assigned NDM-17) identified in a β-lactam-resistant sequence type 48 (ST48) *Escherichia coli* strain that was isolated from a chicken in China. Compared to NDM-1, NDM-17 had three amino acid substitutions (V88L, M154L, E170K) that confer significantly enhanced carbapenemase activity, When compared to NDM-5, NDM-17 had only one amino acid substitution (E170K) and slightly increased isolate resistance toward carbapenem, as indicated by increased MIC values. The gene encoding NDM-17 ($bla_{\text{NDM-17}}$) was located on an IncX3 plasmid, which was readily transferrable to recipient *E. coli* J53 by conjugation, suggesting the possibility of rapid dissemination of $bla_{\text{NDM-17}}$. Enzyme kinetics showed that NDM-17 could hydrolyze all β-lactams tested, except for aztreonam, and had significantly higher affinity for all β-lactams tested compared to NDM-5. The emergence of this novel NDM variant could pose a threat to public health because of its transferability and enhanced carbapenemase activity.

INTRODUCTION

Carbapenem-resistant Enterobacteriaceae (CRE) have been recognized as an urgent antibiotic resistance threat by the Centers for Disease Control in the US, and have become a global problem in recent years (1). The resistance exhibited by CRE is largely mediated by the production of carbapenemases (2), especially metallo-β-lactamases (MBLs) such as VIM, IMP, and New Delhi metallo-β-lactamase (NDM), which can hydrolyze almost all carbapenem β-lactams (3). Since its discovery in India in 2008, NDM has been identified throughout the world, and its identification in China has become common (4, 5). Currently, there are 16 NDM variants (www.lahey.org/studies), with amino acid substitutions at 14 positions. The evolution and spread of NDM are rapid, and NDM-positive bacteria are found in the wider community environment, not just hospitals (6). The spread of NDM-positive bacteria depends on fecal-oral transmission, and an important route for this transmission is animal-derived food (6). The importance of minimizing the carriage of NDM-positive bacteria by food animals for public health was underlined by the discovery of non-human sources of NDM (7,8). In comparison with the high prevalence of NDM-positive Enterobacteriaceae of human origin, there are few reports on CRE from food animals. Furthermore, none of the novel NDM variants described to date were originally identified in bacteria isolated from food animals. Here we describe the characterization of a novel NDM variant in *Escherichia coli* isolated from a chicken.

MATERIALS AND METHODS

Identification and phenotypic characterization of the isolate.

A route annual surveillance of CRE of animal origin was performed to monitor its dissemination. *E. coli* AD-19R was isolated from a cloacal swab taken from a chicken at a commercial poultry farm in Shandong Province, China, in 2015. The sample was plated directly onto CHROMagar KPC selective medium (CHROMagar, Paris, France), which selects for the growth of carbapenem-resistant Enterobacteriaceae (9). The bacterial species was identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker Daltonik, Bremen, Germany), and confirmed by 16S rRNA sequencing (10). The modified Hodge test, using imipenem and meropenem discs, was conducted to confirm the phenotype of carbapenemase production.

Antimicrobial susceptibility testing.

The MICs of the original isolate (AD-19R), its transconjugants and transformants, and two reference isolates (*E. coli* strain YW carrying $bla_{\text{NDM-1}}$ and DZ2-29R carrying $bla_{\text{NDM-5}}$) to several antimicrobials (listed in Table 1) were determined using a broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (11). The *E. coli* ATCC 25922 was used as a quality control strain.

Detection of β-lactamase genes and whole-genome sequencing.

Whole-cell DNA was extracted from isolates AD-19R using a QiaAmp Mini kit according to the manufacturer's recommendations (Qiagen, Hilden, Germany). PCR and

DNA sequencing were conducted to screen for known β-lactamase genes (MBL genes *bla*$_{DIM}$, *bla*$_{GIM}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{SIM}$, *bla*$_{SPM}$, and *bla*$_{VIM}$) as described previously (12). A 150-bp paired-end library was constructed following the standard Illumina (San Diego, CA, USA) paired-end protocol, and the whole genome of *E. coli* AD-19R, including plasmid pAD-19R extracted from transformants, was sequenced on the Illumina HiSeq 2500 system. Results were analyzed using CLC Genomics Workbench version 9.0 (CLC bio, Aarhus, Denmark), and each predicted ORF was used as a query against the GenBank database of the National Center for Biotechnology Information using a BLAST search. Gaps in the sequence were closed by PCR and Sanger sequencing (13).

MLST, Southern blotting, transconjugation, and plasmid analysis.

MLST was performed as described previously to identify the sequence type of isolate AD-19R (14). Southern blot analysis was used with specific *bla*$_{NDM}$ digoxigenin-labeled probes to locate *bla*$_{NDM}$ genes. Transconjugation assays were used to evaluate the horizontal transferability of *bla*$_{NDM}$, with *E. coli* J53 as the recipient, and isolate AD-19R as the donor. The transconjugants were selected on MacConkey agar containing 100 mg/l sodium azide and 1 mg/l meropenem and the transfer frequency was calculated by transconjugants/donors. PCR with specific primers was used to confirm the presumptive transconjugants (15). Plasmid incompatibility groups were determined by two PCR-based replicon typing methods (16,17).

Cloning of *bla*$_{NDM-17}$, *bla*$_{NDM-5}$ and *bla*$_{NDM-1}$.

To compare the beta-lactamase activities of both NDM-1 and NDM-5 with NDM-17, the respective genes (*bla*$_{NDM-1}$, *bla*$_{NDM-5}$ and *bla*$_{NDM-17}$) with their native promoters were amplified by PCR using primers NP-NDM-F (5′-CGGGATCCCACCTCATGTTTGAATTCGC-3′) and NP-NDM-R (5′-CCCAAGCTTCTCTGTCACATCGAAATCGC-3′), and cloned into the pHSG398 vector (Takara Bio, Dalian, China). The resulting plasmids were named pHSG398/NP-NDM-1, pHSG398/NP-NDM-5 and pHSG398/NP-NDM-17, respectively. The complete *bla*$_{NDM-1}$ *bla*$_{NDM-5}$ and *bla*$_{NDM-17}$ ORFs were obtained by PCR using primers NDM-F (5′-CGGGATCCATGGAATTGCCCAATATTATG-3′) and NDM-R (5′-CCCAAGCTTTCAGCGCAGCTTGTCGGCCAT-3′), cloned into pHSG398, and named pHSG398/NDM-1, pHSG398/NDM-5 and pHSG398/NDM-17, respectively. Subsequently, pHSG398/NP-NDM-1, pHSG398/NP-NDM-5, pHSG398/NP-NDM-17, pHSG398/NDM-1, pHSG398/NDM-5 and pHSG398/NDM-17 were transformed into *E. coli* DH5α by electrotransformation, and confirmed by PCR and DNA sequencing (18,19).

Expression and purification of NDM-17 and NDM-5.

The ORFs coding for NDM-5 and NDM-17 without signal peptide regions were amplified using primers BamHI-TEV-NDM-F (5′-ATGGATCCGAAAACCTGTATTTCCAAGGCCAGCAAATGGAAACTGGCGAC-

115 3′) and XhoI-NDM-R (5′ATCTCGAGTCAGCGCAGCTTGTCGGCCATG-3′) and then
116 cloned into the pET28a expression vector (Merck Millipore, Danvers, MA, USA). The
117 resulting plasmid was transformed into *E. coli* BL21 (DE3) as per the manufacturer's
118 instructions (TransGen Biotech, Beijing, China). Ni-nitrilotriacetic acid (NTA) agarose
119 was used to purify the recombinant NDM proteins according to the manufacturer's
120 instructions (Qiagen, Hilden, Germany). His tags were removed by cleaving with Turbo
121 TEV protease (Accelagen, San Diego, CA, USA), and untagged proteins were purified by
122 an additional passage in Ni-NTA agarose. The purity of the recombinant NDM proteins
123 was estimated by SDS-PAGE, and protein concentration was measured using a Pierce
124 bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, MA, USA).
125 β-lactamase activity was monitored with nitrocefin (Oxoid Ltd., Basingstoke, United
126 Kingdom) during the purification procedure, as per the manufacturer's instructions.

127 **Determination of kinetic parameters.**

128 A kinetic study was conducted to measure β-lactamase activity and compare the
129 catalytic properties of NDM-17 and NDM-5. Initial hydrolysis rates were determined in
130 50 mM phosphate buffer (pH 7.0) containing 30 μM Zn^{2+} at 25°C (20), using a
131 SpectraMax M5 multi-detection microplate reader (Molecular Devices, Sunnyvale, CA,
132 USA). The K_m and k_{cat} values and the k_{cat}/K_m ratio were determined from three individual
133 experiments using wavelengths and extinction coefficients as previously described
134 (21,22), and by constructing a Lineweaver-Burk plot.

135 **Accession number(s)**. The sequence of novel NDM variant gene has been deposited
136 in GenBank under accession no. KX812714, and assigned to be *bla*$_{\text{NDM-17}}$ and its
137 BioSample has also been submitted to NCBI. The complete nucleotide sequence of
138 plasmid pAD-19R has been deposited as GenBank accession no. KX833071.

139 **RESULTS AND DISCUSSION**

140 **Characterization of *E. coli* AD19R.**

141 The *E. coli* AD-19R isolate was resistant to all β-lactams tested, including
142 imipenem, meropenem, ertapenem, and aztreonam, but was sensitive to tigecycline and
143 colistin (Table 1). A positive result in the modified Hodge test demonstrated the
144 carbapenemase production phenotype. The presence of *bla*$_{\text{NDM}}$ in AD19R was confirmed
145 by PCR and sequencing. Analysis of the draft genome of AD19R by whole-genome
146 sequencing revealed a novel *bla*$_{\text{NDM}}$ variant, assigned *bla*$_{\text{NDM-17}}$ (GenBank accession no.
147 KX812714), as well as the presence of additional β-lactamase genes*bla*$_{\text{CTX-M-64}}$ and
148 *bla*$_{\text{TEM-1B}}$, sulfonamides resistance gene *sul2* and aminoglycoside resistance genes
149 *aph(3')-Ia*, *aadA5*, *rmtB*. In comparison with *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-17}}$ contained point
150 mutations at nucleotide positions 262 (G→T), 460 (A→C), and 508 (G→A). These
151 substitutions corresponded to amino acid variants V88L, M154L, and E170K,
152 respectively, with the E170K being a novel substitution. Multi-locus sequence typing
153 (MLST) analysis showed that AD19R belonged to sequence type 48 (ST48), which is
154 most commonly associated with *bla*$_{\text{CTX-M}}$-harboring *E. coli* isolates in humans (23).

These findings suggest the possibility of transfer of *E. coli* isolates harboring bla_{NDM} from humans to food animals (8, 24).

Transferability and localization of bla_{NDM-17} and plasmid analysis.

Transconjugation assays showed that bla_{NDM-17} was successfully transferred to *E. coli* J53, with a transfer frequency of ~6.32×10^{-9} per donor. S1-pulsed-field gel electrophoresis and Southern blotting revealed that a plasmid band from the transconjugants (designated AD19/J53), with a size of ~47 kb, hybridized with the bla_{NDM} probe (Fig. 1). AD19/J53 exhibited a similar resistance profile to parental isolate AD19R, except for aztreonam.

The complete DNA sequence of pAD-19R (carrying bla_{NDM-17}), isolated from AD19/J53 transconjugants, was obtained by whole-genome sequencing, with an average depth of coverage of 510. It was a circular, 46,161-bp plasmid with a G+C content of 46.6% and 60 putative open reading frames (ORFs) (Fig. 2). pAD-19R was identified as an IncX3 plasmid, with a typical backbone structure for this plasmid type, including regions involved replication, partitioning, plasmid maintenance, transcriptional activation, and conjugation/type IV secretion (25,26). Although IncX3 plasmids are considered low prevalence, narrow-host-range plasmids of Enterobacteriaceae (27), they may have served as a common vehicle mediating bla_{NDM} dissemination in China, and might be responsible for the rapid spread of NDM-carrying isolates (4,28), a theory supported by our study.

175 BLAST homology analysis showed that pAD-19R had 99% (46142/46161 bp)
176 identity and 100% query coverage with pNDM5_IncX3 (GenBank accession no.
177 KU761328), a 46,161 bp IncX3 plasmid isolated from *Klebsiella pneumoniae* (SZ204),
178 recently reported in China (29). Notably, strain SZ204 carried a *mcr-1*-harboring plasmid,
179 in addition to pNDM5_IncX3, which makes co-dissemination of IncX3
180 $bla_{\text{NDM-5}}$-harboring plasmids and *mcr-1*-harboring plasmids. In addition, the pAD-19R
181 sequence was similar to other five IncX3 bla_{NDM}-allele-harboring plasmids: plasmid
182 unnamed2 from *K. pneumoniae* strain NUHL24835 (GenBank accession no. CP014006)
183 isolated in China, pNDM_MGR194 (GenBank accession no. KF220657) from *K.*
184 *pneumoniae* isolated in India (26), pEc1929 (GenBank accession no. KT824791) from *E.*
185 *coli* isolated in China (30), pJEG027 (GenBank accession no. KF220657) from *K.*
186 *pneumoniae* isolated in Australia (31), and pKpN01-NDM7 (GenBank accession no.
187 CP012990) from *K. pneumoniae* isolated in Canada (32). Interestingly, all six plasmids,
188 including pNDM5_IncX3, were carried by bacteria isolated from humans, whereas the *E.*
189 *coli* strain carrying pAD-19R in our study was isolated from a chicken. This result
190 further indicates the possible transfer of IncX3 $bla_{\text{NDM-17}}$-harboring plasmids/isolates
191 between humans and food-producing animals. Therefore, Enterobacteriaceae species
192 carrying IncX3 $bla_{\text{NDM-17}}$-harboring plasmids should be monitored worldwide.

193 Further analysis of the pAD-19R sequence showed that it didn't harbor other
194 resistance genes apart from $bla_{\text{NDM-17}}$ and *ble*. The sequence surrounding $bla_{\text{NDM-17}}$ shares

195 a common genetic background with a 10,410-bp fragment,
196 Tn*3*-IS*3000*-ΔIS*Aba125*-IS*5*-*bla*$_{NDM-17}$-*ble*$_{MBL}$-*trpF*-*dsbC*-IS*26*-Δ*umnD* (Region A in Fig.
197 S1), which plays a crucial role in horizontal transmission, and may assist in horizontal
198 transfer of *bla*$_{NDM-17}$ among Enterobacteriaceae (33). Overall, these results warn that both
199 the genetic environment of *bla*$_{NDM-17}$ and the IncX3 *bla*$_{NDM-17}$-harboring plasmids
200 contribute to *bla*$_{NDM-17}$ transmission among food-producing animals. The *bla*$_{NDM-17}$
201 carrying isolates would pose a threat to human health once the *E. coli* AD-19R
202 transferred to humans through the food chain, and vice versa.

203 **Functional analysis of NDM-17 and characterization of kinetic parameters.**

204 NDM-17 had three amino acid substitutions (V88L, M154L, E170K) compared
205 with NDM-1, but only one difference (E170K) in comparison with NDM-5, with which
206 NDM-17 shares the closest relationship among the 16 reported NDM variants
207 (www.lahey.org/studies). In order to determine the effects of these amino acid
208 substitutions in NDM-17, especially E170K, cloning experiments and kinetic studies
209 were performed by reference to NDM-5.

210 All of the transformants were successfully cloned and confirmed by PCR. Strains
211 containing pHSG398/NP-NDM-1, pHSG398/NP-NDM-5 and pHSG398/NP-NDM-17,
212 with their native promoters identified no differences for all transformants by PCR and
213 sequencing with M13 primers, exhibited resistance to all β-lactams tested, including
214 meropenem and imipenem (Table 1). Interestingly, the constructs pHSG398/NDM-1,

pHSG398/NDM-5 and pHSG398/NDM-17, carrying complete ORFs without the native promoters, showed reduced susceptibility to penicillins and cephems, but were susceptible to carbapenem. This result confirmed that the wild-type promoter was crucial for carbapenem resistance (19). In addition, all transformants were susceptible to aztreonam, colistin, and tigecycline, which was consistent with previous reports (18,19). The profiles of β-lactams tested resistance for NDM-17 transformants were similar to those for corresponding NDM-5 transformants, however, the MICs of ertapenem and meropenem for pHSG398/NP-NDM-17 were slightly higher (2-fold) than those for pHSG398/NP-NDM-5 (Table 1). Importantly, the MICs of cefepime, ertapenem, and imipenem for DH5α (pHSG398/NP-NDM-17) were 2-fold higher than those of DH5α (pHSG398/NP-NDM-1). Furthermore, DH5α (pHSG398/NP-NDM-17) showed a 4-fold elevation in MIC for meropenem compared with DH5α (pHSG398/NP-NDM-1). These findings suggest that mutations outside the promoter region are responsible for the increased carbapenem resistance.

Expression and purification experiments showed that the NDM-17 and NDM-5 recombinant proteins were expressed at up to 90% purity, as evaluated by SDS-PAGE. Both NDM proteins were used to determine kinetic parameters, which revealed that NDM-17 and NDM-5 could hydrolyze all β-lactams tested, except for aztreonam (Table 2). NDM-17 had similar k_{cat}/K_m ratios for almost β-lactams tested to NDM-5, except for significantly higher k_{cat}/K_m ratios for cefoxitin and penicillin G, and lower for ampicillin.

These results indicate that NDM-17 has similar enzymatic activity to NDM-5, which had been reported to increase the carbapenemase activity compared with NDM-1. Notably, the K_m of NDM-17 for all β-lactams tested was obviously lower than that of NDM-5, especially for ceftazidime, penicillin G, ertapenem, imipenem, and meropenem (Table 2). These results suggest that NDM-17 has significantly higher affinity for all β-lactams tested than NDM-5.

It is possible that the increased resistance and the higher enzyme activity of NDM-17 is conferred by the three amino acid substitutions (V88L, M154L, and E170K). The M154L substitution increases the carbapenemase activity of NDM-4 (M154L) (15), NDM-5 (V88L, M154L) (34-36), and NDM-7 (D130N, M154L) (19,37), indicating it may be responsible for the higher hydrolytic activity of NDM-17. NDM-4 and NDM-5 are identical except for the V88L substitution in NDM-5, and NDM-5 has lower k_{cat}/K_m values for imipenem and meropenem than NDM-4 (38). This suggests that V88L might contribute to the decreased hydrolytic activity of NDM-5 towards carbapenems. NDM-17 shares the V88L and M154L substitutions with NDM-5, in addition to E170K. Our kinetic data showed that NDM-17 had significantly higher affinity for all β-lactams tested, and obviously increased catalytic efficiencies for cefoxitin and penicillin G. Thus, the E170K substitution should be responsible for the higher affinity and increased catalytic efficiencies of NDM-17. Interestingly, the D130G substitution increases carbapenemase activity, but NDM-8which contains both D130G and M154L, does not

exhibit increased hydrolytic activity for carbapenems (39). Thus, it is possible that certain amino acid substitutions may have different effects in different NDM variants, and the increased hydrolytic activity of NDM-17 was not the result of the cumulative effect of the individual V88L, M154L, and E170K amino acid substitutions, but rather the overall interaction of the three substitutions.

To determine the locations of the three amino acid substitutions and analyze their effects on structure, a 3D model of NDM-17 was generated by homology modeling using NDM-1 as a template (PDB accession: 4EXS). The previously reported crystal structure of NDM-1 shows that the active site is formed by loops 3 and 10, at the bottom of a shallow groove, and amino acid triads that bind to zinc ions are formed by H120, H122, and H189, and D124, C208, and H250 (40,41). Currently, 16 amino acid substitutions have been reported in NDMs at 14 distinct amino acids: 28, 32, 36, 69, 74, 88, 95, 130, 152, 154, 200, 222, 233, and 264. E170K represents a new amino acid substitution and site, which was far from the active site and exposed to the solvent. Although positions 88, 154, and 170 are not located in the active site involved in binding to zinc ions (Fig. 3), they might still indirectly affect the formation of the active site, as was previously described (18).

Conclusions.

In this study, a novel NDM variant, NDM-17, was identified in a ST48 *E. coli* strain isolated from a chicken. This is the first report of a new NDM variant being isolated from

a food animal. NDM-17 displayed higher affinity than NDM-5 against almost all β-lactams, as well as carbapenem confirmed by kinetic parameters, and increased carbapenemase activity compared to NDM-1 indicated by MICs. In addition, *bla*$_{\text{NDM-17}}$ was located on an IncX3 plasmid and was surrounded by multiple insertion sequences, mediating the rapid dissemination of *bla*$_{\text{NDM}}$. Transmission of strains carrying *bla*$_{\text{NDM-17}}$ to humans via the food chain represents a serious threat to human health, and should be given further attention to ensure NDM-17-producing pathogens are efficiently monitored.

ACKNOWLEDGMENTS

The study was supported by grants from the National Natural Science Foundation of China (31370046, 31422055 and 8161101130) and the National Basic Research Program of China (2013CB127200).

COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

AUTHORS' CONTRIBUTIONS

Jianzhong Shen designed the study. Zhihai Liu, Dejun Liu, Rongmin Zhang, Jiyun Li and Wenjuan Yin collected the data. Zhihai Liu, Yang Wang, Zhangqi Shen Timothy R. Walsh and Hong Yao analyzed and interpreted the data. Zhihai Liu, Yang Wang, Timothy R. Walsh, Jianzhong Shen wrote the report. All authors revised, reviewed and approved the final report.

295 **REFERENCES**

296 1. **Wu W, Espedido B, Feng Y, Zong Z**. 2016. *Citrobacter freundii* carrying *bla*$_{KPC-2}$
297 and *bla*$_{NDM-1}$: characterization by whole genome sequencing. Sci Rep **6**.30670.
298 2. **Temkin E, Adler A, Lerner A, Carmeli Y**. 2014. Carbapenem-resistant
299 Enterobacteriaceae: biology, epidemiology, and management. Ann N Y Acad Sci
300 **1323**:22–42.
301 3. **Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R,**
302 **Chaudhary U, Doumith M, Giske CG, Irfan S**. 2010. Emergence of a new
303 antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular,
304 biological, and epidemiological study. Lancet Infect Dis **10**:597–602.
305 4. **Feng Y, Yang P, Xie Y, Wang X, McNally A, Zong Z**. 2015. Escherichia coli of
306 sequence type 3835 carrying *bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$, *bla*$_{CMY-42}$ and *bla*$_{SHV-12}$. Sci Rep
307 **5**.12275.
308 5. **Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR**. 2009.
309 Characterization of a new metallo-β-lactamase gene, *bla*$_{NDM-1}$, and a novel
310 erythromycin esterase gene carried on a unique genetic structure in *Klebsiella*
311 *pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother **53**:5046–
312 5054.
313 6. **Wei W, Yang H, Ye Y, Li J**. 2015. New Delhi metallo-β-lactamase mediated
314 carbapenem resistance: origin, diagnosis, treatment and public health concern. Chin
315 Med J (Engl) **128**:1969.

316 7. **Wang Y, Wu C, Zhang Q, Qi J, Liu H, Wang Y, He T, Ma L, Lai J, Shen Z**. 2012.
317 Identification of New Delhi metallo-β-lactamase 1 in *Acinetobacter lwoffii* of food
318 animal origin. PLoS One **7**:e37152.
319 8. **Zhang R, Wang Y, Liu Z, Li J, Yin W, Lei L, Wu C, Shen J**. 2015.
320 Characterization of NDM-1-producing carbapenemase in *Acinetobacter spp.* and *E.*
321 *coli* isolates from diseased pigs. Front Agr Sci Eng **2**:223.
322 9. **Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J**. 2008.
323 Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant
324 *Enterobacteriaceae*. J Clin Microbiol **46**:3110–3111.
325 10. **Suzuki MT, Taylor LT, DeLong EF**. 2000. Quantitative analysis of small-subunit
326 rRNA genes in mixed microbial populations via 5 '-nuclease assays. Appl Environ
327 Microbiol **66**:4605–4614.
328 11. **Clinical and Laboratory Standards Institute**. 2015. Clinical and Laboratory
329 Standards Institute. 2015. Performance standards for antimicrobial susceptibility
330 testing; 25th informational supplement, M100-S25. Clinical and Laboratory
331 Standards Institute, Wayne, PA.
332 12. **Nordmann P, Naas T, Poirel L**. 2011. Global Spread of Carbapenemase-producing
333 Enterobacteriaceae. Emerg Infect Dis **17**:1791–1798.
334 13. **Li D, Wang Y, Schwarz S, Cai J, Fan R, Li J, Feßler AT, Zhang R, Wu C, Shen**
335 **J**. 2016. Co-location of the oxazolidinone resistance genes optrA and cfr on a

multiresistance plasmid from *Staphylococcus sciuri*. J Antimicrob Chemother **71**:1474–1478.

14. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, Achtman M**. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol Microbiol **60**:1136–1151.

15. **Nordmann P, Boulanger AE, Poirel L**. 2012. NDM-4 Metallo-beta-Lactamase with Increased Carbapenemase Activity from *Escherichia coli*. Antimicrob Agents Chemother **56**:2184–2186.

16. **Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A**. 2012. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. Plasmid **68**:43–50.

17. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ**. 2005. I Identification of plasmids by PCR-based replicon typing. J Microbiol Methods **63**:219–228.

18. **Zou D, Huang Y, Zhao X, Liu W, Dong D, Li H, Wang X, Huang S, Wei X, Yan X, Yang Z, Tong Y, Huang L, Yuan J**. 2015. A Novel New Delhi Metallo-β-Lactamase Variant, NDM-14, Isolated in a Chinese Hospital Possesses Increased Enzymatic Activity against Carbapenems. Antimicrob Agents Chemother **59**:2450–2453.

19. **Gottig S, Hamprecht AG, Christ S, Kempf V AJ, Wichelhaus TA**. 2013.

Detection of NDM-7 in Germany, a new variant of the New Delhi metallo-β-lactamase with increased carbapenemase activity. J Antimicrob Chemother **68**:1737–1740.

20. **Li T, Wang Q, Chen F, Li X, Luo S, Fang H, Wang D, Li Z, Hou X, Wang H**. 2013. Biochemical characteristics of New Delhi metallo-β-lactamase-1 show unexpected difference to other MBLs. PLoS One **8**:e61914.

21. **Crowder MW, Walsh TR, Banovic L, Pettit M, Spencer J**. 1998. Overexpression, Purification, and Characterization of the Cloned Metallo-β-Lactamase L1 from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother **42**:921–926.

22. **Queenan AM, Shang W, Flamm R, Bush K**. 2010. Hydrolysis and inhibition profiles of β-lactamases from molecular classes A to D with doripenem, imipenem, and meropenem. Antimicrob Agents Chemother **54**:565–569.

23. **Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Claeys G, Lontie M, Van Meensel B, Herman L, Haesebrouck F**. 2010. Characterization of extended-spectrum β-lactamases produced by *Escherichia coli* isolated from hospitalized and nonhospitalized patients: emergence of CTX-M-15-producing strains causing urinary tract infections. Microb Drug Resist **16**:129–134.

24. **Wang Y, Zhang R, Li J, Wu Z, Yin W, Schwarz S, Tyrrell JM, Zheng Y, Wang S, Shen Z, Liu Z, Liu J, Lei L, Li M, Zhang Q, Wu C, Zhang Q, Wu Y, Walsh TR, Shen J**. 2016. Comprehensive resistome analysis reveals the prevalence of NDM and

376 MCR-1 in Chinese poultry production. Nature microbiology **2**:
377 DOI:10.1038/nmicrobiol.2016.260.
378 25. **Ho P, Li Z, Lo W, Cheung Y, Lin C, Sham P, Cheng VC, Ng T, Que T, Chow K**.
379 2012. Identification and characterization of a novel incompatibility group X3 plasmid
380 carrying *bla*$_{NDM-1}$ in Enterobacteriaceae isolates with epidemiological links to
381 multiple geographical areas in China. Emerg Microbes Infec **1**:e39.
382 26. **Krishnaraju M, Kamatchi C, Jha AK, Devasena N, Vennila R, Sumathi G,**
383 **Vaidyanathan R**. 2015. Complete sequencing of an IncX3 plasmid carrying *bla*$_{NDM-5}$
384 allele reveals an early stage in the dissemination of the *bla*$_{NDM}$ gene. Indian J Med
385 Microbiol **33**:30-38.
386 27. **Yang P, Xie Y, Feng P, Zong Z**. 2014. *bla*$_{NDM-5}$ carried by an IncX3 plasmid in
387 *Escherichia coli* sequence type 167. Antimicrob Agents Chemother **58**:7548–7552.
388 28. **Zhang F, Xie L, Wang X, Han L, Guo X, Ni Y, Qu H, Sun J**. 2016. Further Spread
389 of *bla*$_{NDM-5}$ in *Enterobacteriaceae* via IncX3 Plasmids in Shanghai, China. Front
390 Microbiol **7**:424.
391 29. **Du H, Chen L, Tang Y, Kreiswirth BN**. 2016. Emergence of the mcr-1 colistin
392 resistance gene in carbapenem-resistant Enterobacteriaceae. Lancet Infect Dis
393 **16**:287–288.
394 30. **Chen D, Gong L, Walsh TR, Lan R, Wang T, Zhang J, Mai W, Ni N, Lu J, Xu J**.
395 2015. Infection by and dissemination of NDM-5-producing *Escherichia coli* in

China. J Antimicrob Chemother **71**: 563–565.

31. **Espedido BA, Dimitrijovski B, van Hal SJ, Jensen SO**. 2015. The use of whole-genome sequencing for molecular epidemiology and antimicrobial surveillance: identifying the role of IncX3 plasmids and the spread of *bla*$_{NDM-4}$-like genes in the Enterobacteriaceae. J Clin Pathol **68**:835–838.

32. **Lynch T, Chen L, Peirano G, Gregson DB, Church DL, Conly J, Kreiswirth BN, Pitout JD**. 2016. Molecular evolution of *Klebsiella pneumoniae* ST278 harboring *bla*$_{NDM-7}$ involved in nosocomial transmission. J Infect Dis **214**:798–806.

33. **Wang X, Xu X, Li Z, Chen H, Wang Q, Yang P, Zhao C, Ni M, Wang H**. 2014. An outbreak of a nosocomial NDM-1-producing *Klebsiella pneumoniae* ST147 at a teaching hospital in mainland China. Microb Drug Resist **20**:144–149.

34. **Hornsey M, Phee L, Wareham DW**. 2011. A Novel Variant, NDM-5, of the New Delhi Metallo-β-Lactamase in a Multidrug-Resistant *Escherichia coli* ST648 Isolate Recovered from a Patient in the United Kingdom. Antimicrob Agents Chemother **55**:5952–5954.

35. **Zhu Y, Zhao J, Xu C, Zhao H, Jia N, Li Y**. 2016. Identification of an NDM-5-producing *Escherichia coli* Sequence Type 167 in a Neonatal Patient in China. Sci Rep **6**:29934.

36. **Gamal D, Fernández-Martínez M, El-Defrawy I, Ocampo-Sosa AA, Martínez-Martínez L**. 2016. First identification of NDM-5 associated with

OXA-181 in *Escherichia coli* from Egypt. Emerg Microbes Infec **5**:e30.

37. **Cuzon G, Bonnin RA, Normann P**. 2013. First Identification of Novel NDM Carbapenemase, NDM-7, in *Escherichia coli* in France. PLoS One **8**:e61322.

38. **Makena A, Brem J, Pfeffer I, Geffen REJ, Wilkins SE, Tarhonskaya H, Flashman E, Phee LM, Wareham DW, Schofield CJ**. 2015. Biochemical characterization of New Delhi metallo-beta-lactamase variants reveals differences in protein stability. J Antimicrob Chemother **70**:463–469.

39. **Tada T, Miyoshi-Akiyama T, Dahal RK, Sah MK, Ohara H, Kirikae T, Pokhrel BM**. 2013. NDM-8 Metallo-beta-Lactamase in a Multidrug-Resistant *Escherichia coli* Strain Isolated in Nepal. Antimicrob Agents Chemother **57**:2394–2396.

40. **Green VL, Verma A, Owens RJ, Phillips SEV, Carr SB**. 2011. Structure of New Delhi metallo-β-lactamase 1 (NDM-1). Acta Crystallogr Sect F Struct Biol Cryst Commun **67**:1160–1164.

41. **Zhang H, Hao Q**. 2011. Crystal structure of NDM-1 reveals a common beta-lactam hydrolysis mechanism. FASEB J **25**:2574–2582.

FIGURE LEGENDS

FIG 1. Location of *bla*$_{NDM-17}$ on *E. coli* plasmid AD-19R, separated by PFGE. Lane A, hybridization of the plasmid with a probe specific for *bla*$_{NDM-17}$; lane B, plasmid from transconjugants AD19/J53; lane C, reference standard strain H9812 restricted by *Xba*I.

FIG 2. Homology model of NDM-17. (A) Protein backbone of NDM, shown as a cartoon with the helices and strands. Amino acids binding to zinc ions (H120, H122, H189; D124, C208, H250) and three amino acid substitutions (L88, L154, and K170) are labeled and colored blue and red, respectively. (B) The three amino acid substitutions (red) were not located at the active sites (loop 3 (green) or loop 10 (green)), or near the amino acids binding to zinc ions (blue).

443 **TABLE 1** β-lactam MICs for the NDM17-carrying original *E. coli* isolate, and its transconjugants and transformants

Antibiotic	AD19 (NDM-17)	AD19/J53	J53	DH5α (pHSG398)	DH5α (pHSG398/ NDM-1)	DH5α (pHSG398/ NDM-5)	DH5α (pHSG398/ NDM-17)	DH5α (pHSG398/ NP-NDM-1)	DH5α (pHSG398/ NP-NDM-5)	DH5α (pHSG398/ NP-NDM-17)
Ampicillin	>256	>256	4	2	>256	>256	>256	>256	>256	>256
Aztreonam	256	0.063	0.063	0.032	0.063	0.032	0.063	0.063	0.032	0.063
Amikacin	>256	2	2	0.5	0.5	0.25	0.5	0.5	0.25	1
Cefepime	>256	>256	0.063	0.032	2	2	4	8	16	16
Cefotaxime	>256	>256	0.125	0.063	64	32	32	128	128	128
Cefoxitin	>256	>256	8	4	>256	128	>256	>256	>256	>256
Ceftazidime	>256	>256	0.5	0.25	>256	>256	>256	>256	>256	>256
Ciprofloxacin	16	≤0.008	≤0.008	0.016	0.016	0.008	≤0.008	0.016	0.008	0.016
Colistin	1	0.5	0.5	0.125	0.125	0.016	0.125	0.125	⩽0.008	0.125
Ertapenem	256	128	0.032	0.016	0.25	2	2	64	64	128
Gentamicin	256	0.5	0.5	0.125	0.125	0.125	0.125	0.063	0.063	0.125
Imipenem	128	32	0.5	0.5	2	2	2	8	16	16
Meropenem	128	32	0.063	0.031	1	2	2	8	16	32
Penicillin G	>256	>256	64	32	>256	256	>256	>256	>256	>256
Tigecycline	0.063	0.063	0.063	0.032	0.032	0.032	0.032	0.063	0.016	0.063
SXT (1/19)	≥16/304	0.063/1.2	0.032/0.61	0.5/9.5	0.5/9.5	0.25/4.75	0.25/4.75	0.032/0.61	0.032/0.61	0.063/1.2

444 **TABLE 2** Kinetic parameters of NDM-17 and NDM-5 enzymes[a]
445

	NDM-17[b]			NDM-5[b]			k_{cat}/K_m (μM^{-1} s^{-1})ratio
β-lactam	K_m (μM)	k_{cat} (s^{-1})[b]	k_{cat}/K_m (μM^{-1} s^{-1})	K_m (μM)	k_{cat} (s^{-1})[b]	k_{cat}/K_m (μM^{-1} s^{-1})	for NDM-17/NDM-5
Ampicillin	586±53	157±11	0.27	590±57	267±8.1	0.45	0.60
Aztreonam	NH[c]	NH	NH	NH	NH	NH	NH
Cefepime	81±5.5	7.5±1.76	0.092	102±7.9	11±2.8	0.11	0.83
Cefotaxime	11±2.5	11±3.9	1.00	22±5.4	21±5.9	0.95	1.05
Cefoxitin	23±3.1	5.2±0.04	0.23	45±0.81	6.6±0.47	0.15	1.53
Ceftazidime	82±8.6	10±1.1	0.12	155±16	21±0.76	0.14	0.86
Ertapenem	237±25	49±2.6	0.21	571±20	120±7.5	0.21	1.00
Imipenem	188±0.28	79±2.5	0.42	396±4.3	148±0.64	0.37	1.14
Meropenem	453±33	127±15	0.28	659±36	222±48	0.34	0.82
Penicillin G	365±33	115±13	0.32	660±21	93±16	0.14	2.29

446 [a]The proteins were initially modified with a His tag, which was removed after purification.

Antimicrobial Agents and Chemotherapy

[b] K_m and k_{cat} values are means ± standard deviations from three independent experiments.

[c] NH denotes no hydrolysis under conditions with substrate concentrations up to 1 mM, and enzyme concentrations up to 700 nM.

FIG 1

FIG 2